Methotrexate in the Treatment of Malignant Tumours:
Evidence for the Possible Participation of Host Defence Mechanisms

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THE anti-folic acid drug methotrexate is of value in the treatment of acute lymphoblastic leukemia in children and in choriocarcinoma of women.[1] It has also been used successfully in the treatment of Burkitt's lymphoma,[2] and there is now evidence that it is of temporary value in the treatment of solid tumours, particularly epidermoid carcinoma of the head and neck.[3]

The case history of a male patient with epidermoid cancer of the lung prompted a review of those patients in the city of Ottawa who had received methotrexate for treatment of malignant tumours.

PATIENTS AND METHODS

Seventeen patients attending the Ontario Cancer Foundation Clinics at the Ottawa General Hospital and the Ottawa Civic Hospital, who had received methotrexate, make up the group studied. Preparation of tumour material for skin testing and analysis of the cellular stromal reaction in tumour biopsies have been described previously in detail, as has the preparation of Varidase (Lederle), a commercial mixture of streptokinase and streptodornase, and the patient's white cells as controls.[4, 5] Endotoxin was supplied by Dr. E. Ribi, Montana.

CASE REPORT

The patient, R.B., was a 52-year-old white man. He was illiterate, married but childless, and was employed as a trash collector. He was admitted to hospital on June 6, 1968, with a three-month history of weakness in the right arm, pain in the right shoulder and gradual swelling of the tissues of the right thorax, breast, neck and face. He had a two-week history of cough, producing greenish sputum. His history was remarkable only in that he had smoked 25 cigarettes a day for 42 years.

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This work was supported in part by the Ontario Cancer Treatment and Research Foundation Grant No. 168.
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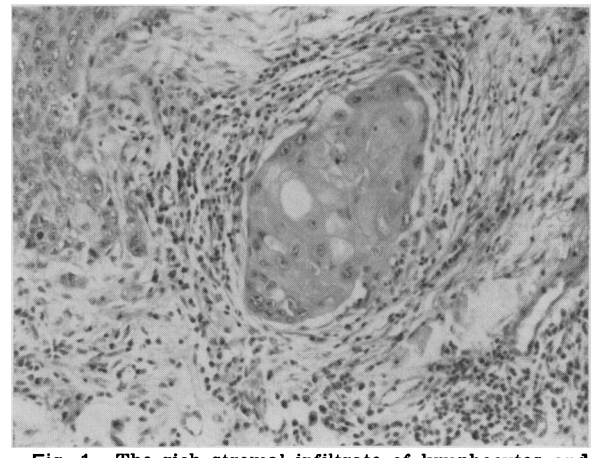

Fig. 1.—The rich stromal infiltrate of lymphocytes and plasma cells in this epidermoid carcinoma of lung, metastatic to subcutaneous tissue, on June 7, 1968. (H. and E., × 150.)

Physical examination showed a well-nourished middle-aged man with obvious swelling of the soft tissues of the neck and face. His facies was eunuchoid. Engorgement of the superficial neck veins was present and there were many distended veins overlying the upper thorax on both sides, more pronounced on the right. A mass of matted glands was felt on the right side of the neck. A left supraclavicular node, firm and not tender, measured 1 x 2 cm. A hard node, 1.5 cm. in diameter, was felt in the left axilla. The entire right hemithorax was indurated, swollen and dusky red in colour, and over its anterior aspect many small, firm, 1-cm. intradermal nodules were felt. The induration extended across the midline to involve the left breast. The

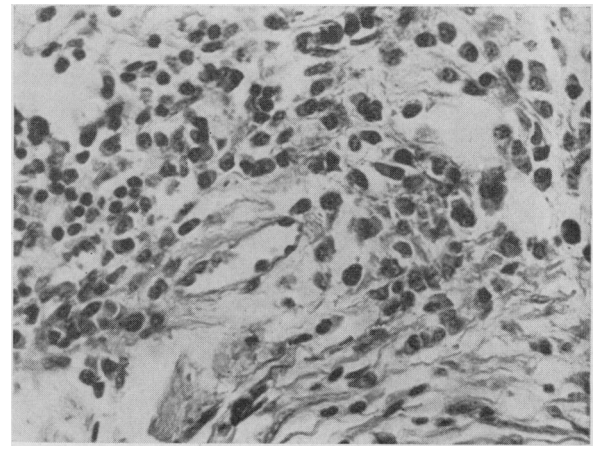

Fig. 2.—Higher-power view of the mononuclear infiltrate shown in Fig. 1. (H. and E., × 260.)

TABLE I.—THE TREATMENT GIVEN TO THE PATIENT R.B. ON EACH OF HIS HOSPITAL ADMISSIONS, WITH RELEVANT LABORATORY STUDIES

Period	*Radiotherapy*	*Antigen stimulus*	*Chemotherapy*	*Laboratory studies*		
June 6, 1968 to July 6, 1968	June 12 to 20, 1968 Deep x-ray seven treatments 1424 R. central dose 2053 R. total dose June 20-July 4, 1968 Cobalt therapy 2000 R. central dose 2200 R. maximum skin dose	June 12 to 22, 1968 Varidase (10,000 units) daily intradermally and subcutaneously Endotoxin I.V. 0.2 μg. June 12 0.3 μg. June 13 0.4 μg. June 14 0.5 μg. June 15 0.7 μg. June 21 0.8 μg. June 22	July 4 to 14, 1968 Methotrexate 1.25 mg. q.i.d. Total dose 50 mg.		High	Low
				Hematocrit	37%*	28%
				WBC	12,000/mm³	3400/mm³
				Lymphocyte count	2400/mm³	330/mm³
				The low lymphocyte count was seen from July 10 to July 26.		
				*Post transfusion		
Sept. 11, 1968 to Nov. 13, 1968	None	Sept. 18, 1968 to Oct. 3, 1968 Eleven daily injections of Varidase, 10,000 units intradermally and subcutaneously Endotoxin I.V. 0.3 μg. Sept. 9 0.7 μg. Sept. 30	Oct. 5 to 8, 1968 Methotrexate 1.25 mg. q.i.d. Total dose 21.25 mg. Oct. 22 to 27, 1968 Methotrexate 1.25 mg. q.i.d. Total dose 25 mg.		High	Low
				Hematocrit	32%	25%
				WBC	7800/mm³	4300/mm³
				Lymphocyte count	2046/mm³	1083/mm³
				Platelet counts did not fall below 218,000/mm.³		
Nov. 30, 1968 to Jan. 3, 1969	None	Nov. 20, 1968 Varidase 10,000 units	Dec. 3 to 8, 1968 Methotrexate 1.25 mg. q.i.d. Total dose 25 mg. Dec. 30, 1968 to Jan. 3, 1969 Methotrexate 1.25 mg. q.i.d. Total dose 21.25 mg.		High	Low
				Hematocrit	32%	25%
				WBC	9400/mm³	3500/mm³
				Lymphocyte count	1692/mm³	420/mm³

right axillary contour was obliterated by induration and there was brawny edema of the right arm. Moderate gynecomastia was present, and the testes were small on palpation. The remainder of the physical examination was negative.

On the day following admission a biopsy was performed under local anesthesia. A wedge of skin and subcutaneous tissue 5 x 1.5 cm. was removed from the chest wall, lateral to the right breast. A portion of this material was given to the pathologist for histological identification and showed metastatic epidermoid carcinoma. A marked stromal reaction of lymphocytes and plasma cells was seen in all the sections (Figs. 1 and 2). The remainder of the tissue was processed by homogenization, sonification and differential centrifugation. Pellets for skin testing were prepared from the material obtained following 2000 G. and 14,000 G. centrifugation for 20 minutes and one hour respectively. Skin tests were performed on June 10. The following materials were used: 0.1 ml. of each of the pellets mentioned; 0.1 ml. of the supernate resuspended in 3.0 ml. of normal saline; endotoxin, 0.01 μg. in 0.1 ml.; Varidase, 0.1 ml. of a solution containing streptokinase and streptodornase, 25 and 10 units of each respectively. Controls used were saline 0.1 ml. and the patient's own white cells, prepared from the buffy cream, 0.1 ml. All injections were given intradermally into the flexor aspect of the left arm. At 45 minutes after the injection faint erythema, 10 x 10 mm., was seen at the site of the two tumour pellet injections; the other injection sites showed no reaction. At 24 hours there was erythema and marked induration, measuring 12 x 12 mm., at the site of injection of the 2000 G. pellet, erythema and slight induration, 10 x 10 mm., at the site of injection of the 14,000 G. pellet, marked erythema and moderate induration, 15 x 15 mm., at the Varidase site and marked erythema and slight induration, 25 x 20 mm., at the site of the endotoxin injection. The sites of injection of saline, the white cells and the supernatant were all negative. The induration and erythema faded over the next 48 hours. It was considered that the patient had a delayed hypersensitivity reaction (DHR) to acellular extracts of his tumour. Bacterial culture of the tumour on a blood agar plate showed no growth after 48 hours and a very light growth of micrococcus after 72 hours of culture in sodium thioglycollate medium. This was considered to be a contaminant. In view of this presumptive evidence of a delayed hypersensitivity reaction, the patient was given injections of Varidase, initially intradermally and later subcutaneously, plus intravenous endotoxin. These antigenic stimulants were given with the aim of accelerating the DHR[6] and completing the final common pathway of the incomplete DHR.[5] Table I summarizes the treatment schedule.

A chest radiograph on June 7 showed homogeneous shadowing in almost all the right upper zone, contiguous with the mediastinum (Fig. 3). This was interpreted as a large tumour mass originating in the lung. Cytological examination of the sputum was positive for undifferentiated tumour

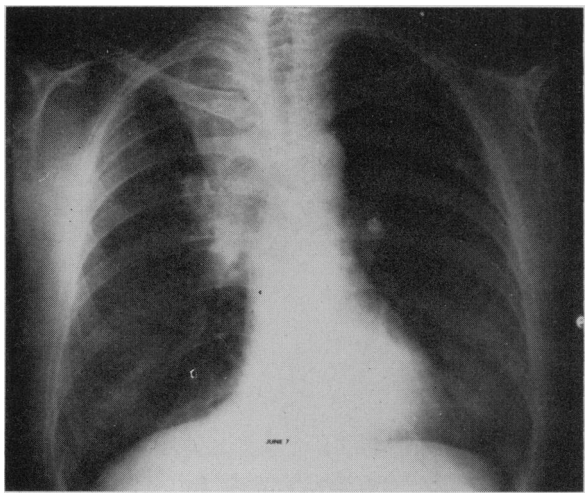

Fig. 3.—Chest radiograph taken on admission. The upper segment tumour shadow is clearly seen on the right.

cells. Chromosome analysis of leukocytes suggested that the patient was a case of mosaic Klinefelter's syndrome.

Since there were signs of superior mediastinal obstruction, deep x-ray therapy was started on June 12 and continued until June 20, by which time a total dose of 2052 R. had been given to the right upper anterior and posterior chest and mediastinum, over an area 10 x 15 cm. The central tumour dose was 1424 R. On June 20 cobalt-60 therapy was begun to a wider area, including the induration of the right chest wall, the biopsy scar and the right supraclavicular area. By July 4 he had received a central tumour dose of 2000 R. Slight relief in the swelling of the neck was noted, but little change had occurred in the extensive infiltration over the right chest wall. The nodes in the left supraclavicular area and left axilla remained unchanged, since they lay outside the field of irradiation. A chest radiograph on July 6 showed a pleural effusion on the right (Fig. 4); the tumour mass in the upper lung field was smaller.

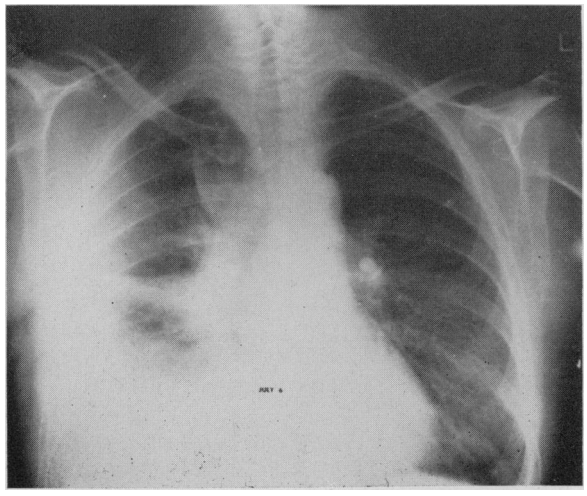

Fig. 4.—Chest radiograph taken at the conclusion of radiotherapy. A right-sided pleural effusion is present. The tumour mass is smaller.

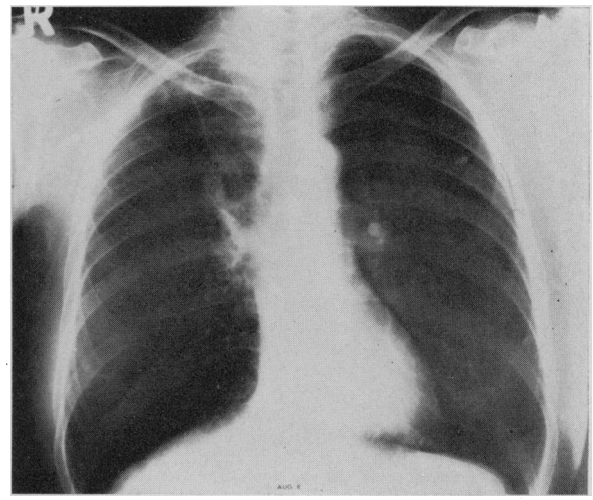

Fig. 5.—Radiograph taken on August 8 showing dramatic improvement following the initial course of methotrexate.

Because of the unsatisfactory response to irradiation, oral methotrexate was started, 1.25 mg. every six hours. He received a total dose of 50 mg. over 11 days, by July 15. Two days later, improvement was noted, with softening of the chest induration and reduction of the swelling of the neck. By July 26 it was evident that his tumour was undergoing marked regression. The matted glands in his neck became smaller and softer and his sense of well-being increased. He was discharged on July 26. A radiograph taken on August 8 confirmed the striking improvement (Fig. 5). He felt well, had gained 10 lbs. in weight and had approximately an 80% regression of the subcutaneous and lymph node tumour. By August 29 there were signs of recurrence. Two intradermal deposits had developed since his discharge from hospital and were clearly visible on the left side of his neck lying adjacent to each other, each measuring 0.75 cm. in diameter. He was readmitted for further treatment on September 9. It was decided to evaluate the use of methotrexate

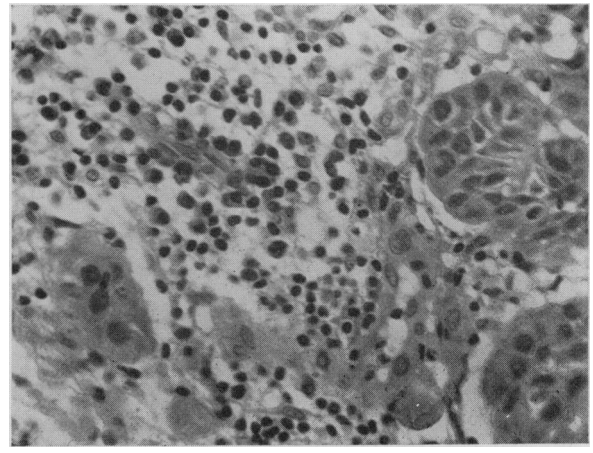

Fig. 6.—The persistence of the stromal infiltrate of lymphocytes and plasma cells in the tumour biopsy of September 17, taken from the same site as the initial biopsy. (H. and E., × 260.)

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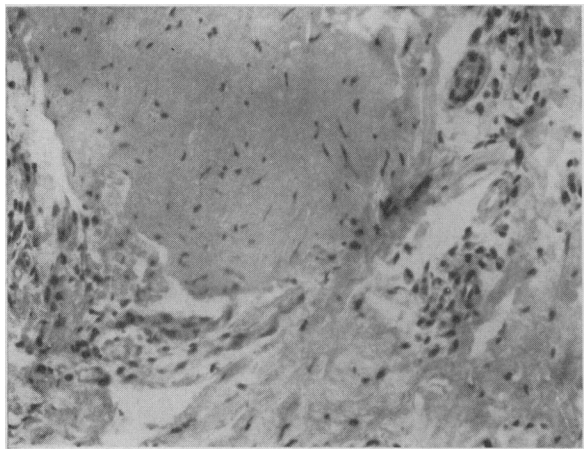

Fig. 7.—Reaction at the site of injection of the tumour pellet suspension following 14,000 G. centrifugation. The biopsy was taken 40 hours after injection. Note the amorphous mass, representing some of the injected material, with adjacent round-cell infiltrate and capillaries whose endothelial cells show prominence. This picture is compatible with a mild delayed hypersensitivity reaction. (H. and E., × 200.)

without radiotherapy and to assess its effect on lesions that had not been irradiated during the first admission. A second biopsy was taken from the site of the initial biopsy on September 17. This area had been included in the cobalt-60 irradiation. The tumour was present and showed a moderate stromal reaction with lymphocytes and plasma cells (Fig. 6), not so pronounced as in the pre-treatment biopsy. Skin testing was repeated, using the 14,000 G. material prepared three months before, which had been stored in a refrigerator at –20° C. There was a minimal reaction of 6 mm. of erythema with induration at 24 hours; Varidase showed a strong reaction, 25 x 30 mm. of erythema and induration. At 40 hours the site of the tumour skin reaction was biopsied (Fig. 7). The dermal capillaries showed a slight reaction with prominence of endothelial cells and a sparse infiltrate of lymphocytes, plasma cells and other mononuclear cells in the perivascular tissues. A further course of Varidase intradermally and subcutaneously and endotoxin intravenously was

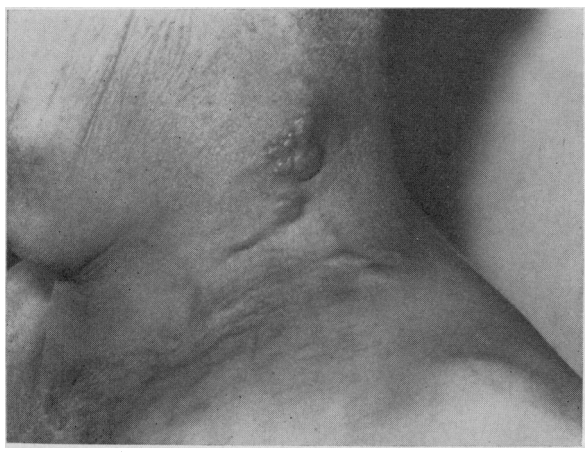

Fig. 8.—The intradermal metastasis on September 17, before treatment with methotrexate.

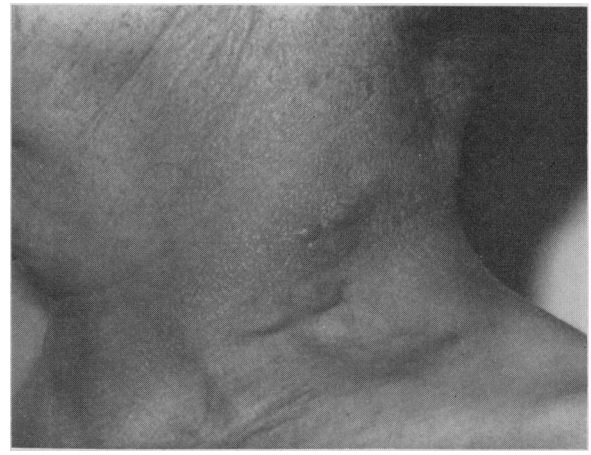

Fig. 9.—By October 15, marked reduction in tumour size is evident, seven days after the first of two courses of methotrexate.

given (Table I). On October 5 methotrexate was given for a total dose of 25 mg., by the split-dose regimen, 1.25 mg. four times daily. Varidase and endotoxin were resumed on October 15 and continued until October 21, when a further course of methotrexate, total dose 20 mg., was given over five days. No adverse effects from the methotrexate were noted. In particular, there was no oral ulceration, vomiting or diarrhea. There was moderate leukopenia and lymphocytopenia but no thrombocytopenia. The patient had a normochromic normocytic anemia throughout the period in hospital and this was attributed to the presence of the tumour. A good regression was obtained in the skin lesion (Figs. 8, 9 and 10 show the effects of the two courses of methotrexate during this second admission). There was no noticeable effect from the injections of Varidase and endotoxin, and while the patient suffered no ill effects, aside from pyrexia and local discomfort at the site of the Varidase injection, there was no measurable effect on the tumour. A noticeable decrease in the size of the neck lesion was seen on October 13, eight days after methotrexate was started and three days after the last

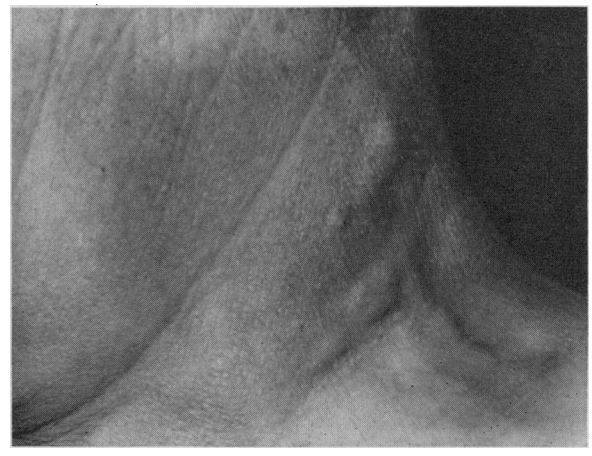

Fig. 10.—Further improvement is seen on November 7, 11 days after the second course of methotrexate.

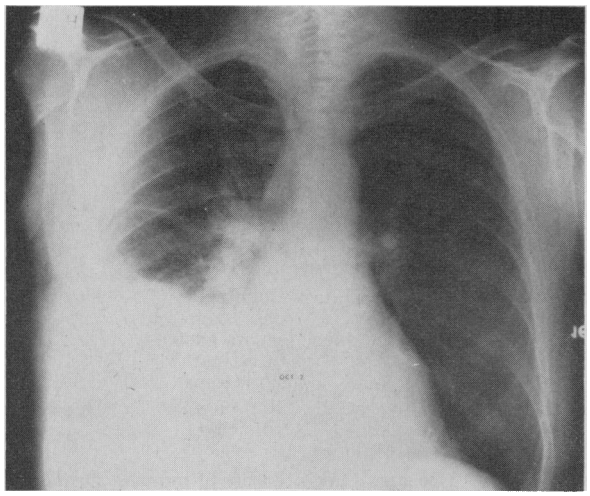

Fig. 11.—Radiograph taken on October 2 shows the reappearance of a right-sided pleural effusion.

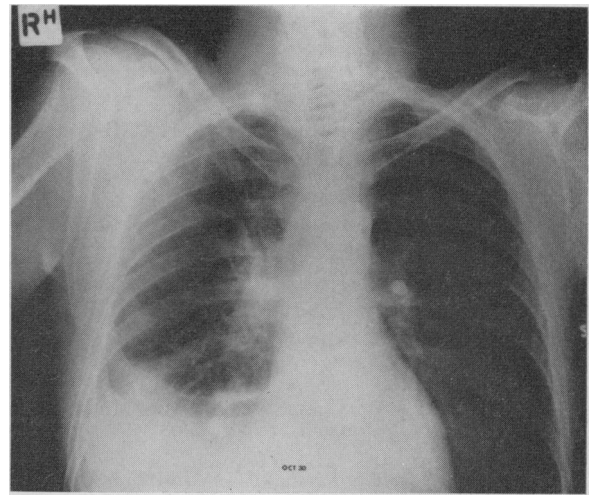

Fig. 12.—Reduction in the amount of effusion following methotrexate. Thoracentesis was not performed.

dose. By October 15 considerable shrinkage of the tumour had occurred (Fig. 9). At this time the recurrent infiltration over the right chest wall was less, with loosening of the subcutaneous tissues. Improvement was also seen in the chest radiographs taken on October 2 and October 30 (Figs. 11 and 12). Over this period the patient's right arm showed complete resolution of the brawny edema, indicating clearing of lymphatic obstruction.

At his request, the patient was discharged on November 13, weighing 116 lbs. He was readmitted on November 30 as there had been deterioration; his weight had fallen to 106 lbs. and he had exacerbation of his cough, an increased number of intradermal nodules and swelling of the right side of his chest with recurrent pain in his right shoulder. A third biopsy was taken from the site of the other two biopsies on December 19. No inflammatory reaction was seen in the stroma of this tissue, again shown to be metastatic epidermoid carcinoma. Another course of methotrexate was given from December 3 to December 8, for a total dose of 25 mg. A transient thrombocytopenia occurred after this and the patient had a persistent lymphocytopenia during this final admission. No significant loss of hair occurred. Slight subjective benefit resulted from the methotrexate, but there was no measurable decrease in tumour size. The patient died on January 3, 1969. An autopsy was performed and the final diagnosis was an anaplastic epidermoid carcinoma arising from the right upper lobe bronchus, metastasizing extensively to the mediastinum, right lung, thyroid, left lung, brain and soft tissues of the thorax. Microscopic examination of multiple sections of the primary tumour site in the lung and the metastatic lesions showed a very sparse lymphocyte and plasma cell stromal reaction. An exception to this was a metastatic deposit in the brain which showed a moderate reaction of these round cells, and this presumably reflected the known inability of methotrexate to cross the blood brain barrier in effective concentration, thus sparing these lymphocytes from the immunosuppressive effect of methotrexate. The spleen was small and atrophic, weighing 75 g. The testes were atrophic and histologically were compatible with Klinefelter's syndrome.

COMMENTS

The course of this patient, from diagnosis to death, does not differ from the general behaviour of cases of solid tumour that have shown regression with treatment using methotrexate.[3, 7, 8] This has been an initial response, sometimes dramatic, with eventual resistance to treatment and death of the patient. To date no explanation has been given that allows one to predict which tumours will respond to methotrexate. *In vitro* studies have shown that cell lines derived from normal and malignant tissues are equally susceptible to the toxic effects of methotrexate.[9] The traditional view that tumours which have a large need for folic acid are those that will respond best has been questioned.[10, 11]

The use of Varidase and endotoxin in this patient was based on the assumption that he showed some evidence of a cell-mediated immunologic defence against the tumour. It has been suggested that patients who have a positive DHR to their tumour, plus a rich stromal infiltration of their tumour by lymphocytes and plasma cells, show a partial but incomplete defence reaction.[5] The aggregation of these cells is suggestive of what occurs *in vitro*, where the first step in the destruction of target cells by sensitized lymphocytes is aggregation around such cells.[12] The second step, destruction of the cell by means that are not yet clear, presumably is not occurring, or is occurring only ineffectively. A full discussion of this concept is given in the paper by Stewart.[5] No beneficial effect was seen from the systemic injection of two antigenic agents, endo-

TABLE II.—SUMMARY OF THE CASES TREATED WITH METHOTREXATE, SHOWING THE RESPONSE TO TREATMENT AND THE DEGREE OF STROMAL INFILTRATE OF THE TUMOUR WITH MONONUCLEAR CELLS

Case No.	*Age*	*Sex*	*Pathological diagnosis*	*Date of biopsy*	*Stromal infiltrate*	*Response to methotrexate*	*Dose of methotrexate*	*Toxicity of methotrexate*	*Present status*
1	66	F	Carcinoma of endometrium with peritoneal metastases	1952 1960	Minimal Minimal	Apparent control of ascites	1.25 mg. t.i.d. orally 6 days. 7 monthly courses	Ulcers of mouth and general malaise	Alive
2	44	F	Carcinoma of breast	1965	Minimal	No response	1.25 mg. q.i.d. 5 days. 5 monthly courses	Ulcers of mouth and general malaise	Dead
3	58	M	Carcinoma of pancreas with hepatic metastases	1968	Minimal	No response	1.25 mg. t.i.d. 10 days. Repeated once	Bone marrow depression and severe gastro-intestinal symptoms	Dead
4	66	M	Carcinoma of stomach	May 1968	Minimal	No response	1.25 mg. q.i.d. 10 days. Repeated once	Nausea and vomiting	Dead
5	32	F	Carcinoma of stomach	April 1968	Moderate	Control of ascites and vomiting. Felt well for three months	1.25 mg. q.i.d. 5 days. 6 courses	Ulcers of mouth, nausea	Dead
6	36	M	Epidermoid carcinoma of hypopharynx with metastasis to the femur	April 1968	Moderate	50% reduction in tumour. Drop in fever; rise in Hb. from 7 to 12 g.	1.25 mg. q.i.d. 7 days. 50 mg. I.V. weekly. 6 courses	Ulcers of mouth	Alive
7	41	M	Mixed epidermoid and oat-cell carcinoma of lung	June 1967	Marked	50% reduction in hepatic metastases	1.25 mg. q.i.d. 9 days. Repeated once	Ulcers of mouth	Dead
8	66	M	Epidermoid carcinoma of alveolus	April 1968 June 1968	Tiny biopsy. One focus of plasma cells and lymphocytes Minimal	90% reduction in tumour	50 mg. I.V. weekly for 22 days. Leucovorin	Liver toxicity. Bone marrow depression	Alive
9	43	M	Epidermoid carcinoma of floor of mouth	October 1967	Moderate to marked	50% reduction in tumour	50 mg. I.V. daily for 45 days. Leucovorin	Severe skin rash	Dead
10	48	M	Epidermoid carcinoma of antrum	April 1968	Minimal	25% reduction in tumour. Relief of pain	50 mg. I.V. for 7 days	Bone marrow depression. Liver toxicity	Alive
11	21	F	Chorio-carcinoma	Curettage 1967 Hysterectomy 1968	Interpretation impossible Moderate to marked	Dramatic response with reduction in tumour size and fall in chorionic gonadotrophins	25 mg. orally for 5 days. 3 courses.	Ulcers of mouth. Bone marrow depression	Alive
12	21	F	Chorio-carcinoma	Spinal cord metastasis 1968 Autopsy 1968	Absent Minimal	No response	25 mg. I.V. daily for 5 days. Repeated once	Bone marrow depression. Ulcers of mouth	Dead
13	22	M	Malignant teratoma of testis	1968	Minimal	No response	1.25 mg. q.i.d. for 6 days	Ulcers of mouth	Alive
14	44	F	Carcinoma of rectum	Primary 1965 Metastasis to perineum 1966 1967 1968	Marked Moderate Minimal Absent	No response	1.25 mg. q.i.d. for 5 days. Course repeated	Ulcers of mouth	Alive

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Case No.	*Age*	*Sex*	*Pathological diagnosis*	*Date of biopsy*	*Stromal infiltrate*	*Response to methotrexate*	*Dose of methotrexate*	*Toxicity of methotrexate*	*Present status*
15	67	F	Carcinoma of breast	Primary 1966	Marked		1.25 mg. q.i.d. for 5 days	None	Alive
				Scar nodule 1968	Minimal				
				1968	Minimal	No response			
16	67	M	Adeno-carcinoma of lung	August 1968	Moderate	No response December 1968	1.25 mg. q.i.d. for 6 days	None	Dead

toxin and Varidase, and this agrees with the observation of Coley[13] that his mixed bacterial toxins were ineffective in causing regression in tumours other than sarcoma. The recent claim of Svet-Moldavsky and Kadaghidze[14] that methotrexate potentiates the toxic effects of lymphocytes against target tumour cells is immediately relevant, as it permits an explanation of both the selective action of this drug in the case described and the eventual resistance of the tumour when the stromal lymphocytic infiltrate became so scanty that no further synergistic effect was obtained. Can one predict which tumours will respond to methotrexate? To determine if this could be done, histological sections from the tumours of 10 patients who had been treated with methotrexate at the Ottawa Civic Hospital were examined by one of us (T.H.M.S.) without a knowledge of clinical details.

REVIEW OF LYMPHOCYTIC AND PLASMA CELL STROMAL RESPONSE IN TUMOURS OF PATIENTS WHO HAD RECEIVED METHOTREXATE

The slides were graded, in terms of lymphocyte and plasma cell infiltrate, as "absent", "minimal", "moderate" and "marked". The slides from two patients showed a dense stromal reaction to the tumour and in each there had been a good response to methotrexate (Cases 7 and 9, Table II). Slides from the remaining eight cases showed a minimal stromal reaction. Four of these patients had no response to the drug (Cases 2, 3, 4 and 12). The available sections of uterine curettings from one woman with choriocarcinoma were difficult to interpret (Case 11). A later examination of slides prepared from the uterus after hysterectomy showed a moderate to marked infiltrate by lymphocytes. In one patient (Case 8) the material available was from a biopsy taken after high-dose perfusion of the carotid artery; the pre-infusion biopsy was very small and no interpretation could be made. In two patients there was improvement in performance, with relief of pain and slight tumour regression in one (Case 10) and apparent control of disease in the other (Case 1). These last three cases have been scored as a failure of correlation between the stromal infiltrate and response to methotrexate.

A further six patients have been studied; these, with the patient R.B., make a total of 17. Three of these (Cases 13, 14 and 15) had no response to methotrexate and had minimal stromal infiltrate of the biopsy taken before treatment. Two patients (Cases 5 and 6) showed a good response to methotrexate and had a moderate stromal infiltrate of the pretreatment biopsy. One patient (Case 16) had a moderate to marked stromal infiltrate of his tumour removed four months before receiving methotrexate, but he had no response at all to the drug. However, for three months before methotrexate he had received prednisone 15 mg. daily in divided doses, following cyclophosphamide and nitrogen mustard. There is a real possibility that this regimen caused effective immunosuppression.

A summary of each patient's course is provided in Table II, which sets forth a grading of available histological material for lymphocyte and plasma cell infiltrate, and an assessment of the patient's response to methotrexate, gauged either as a 50% regression of measurable lesions or as a significant improvement in performance and well-being.

DISCUSSION

In the 16 cases so far studied we made a prediction as to whether the patient would show a response to methotrexate. In 12 instances our prediction proved correct (Table III).

If it is suggested that methotrexate kills tumour cells by its effect on both the tumour cells and adjacent lymphocytes, then one must suppose that the lymphocytes contain material toxic for the adjacent tumour cells. Ruddle and Waksman[15] have shown that sensitized lymphocytes, when confronted with the sensitizing antigen, release cytotoxic factors within hours, and that this precedes lymphocyte transformation by days. Thus in patients who have a rich stromal lymphocyte reaction one would have to postulate that these are cells sensitized and attracted to

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TABLE III.—CORRELATION BETWEEN THE RESPONSE TO METHOTREXATE AND THE DEGREE OF STROMAL INFILTRATE OF THE TUMOUR BY LYMPHOCYTES AND PLASMA CELLS. THE PATIENTS ARE INDICATED BY THEIR NUMBER IN THE CASE SUMMARIES.

Lymphocyte and plasma cell stroma infiltrate	*No response*	*Response*
Absent or minimal	2, 3, 4, 12, 13, 14, 15	1, 8,* 10
Moderate or marked	16	5, 6, 7, 11, 17, (R.B.) 9†

*The pre-infusion biopsy was tiny and interpretation of stromal infiltrate was impossible.

†This patient had a submandibular metastasis. The biopsy showed no lymph node tissue, and the stroma showed areas with lymphocytes and plasma cells. The biopsy was distorted by trauma, and the possibility that the round cells were derived from an adjacent lymph node is considered.

tumour antigen, but, for some reason, release of cytotoxic factors does not occur. Hellström *et al.*[16] have shown that a patient's lymphocytes will kill an autochthonous tumour *in vitro* while there is progressive growth of the tumour *in vivo*. With activation of the lymphocytes following exposure to methotrexate this released cytotoxic factor would be sufficient to destroy the methotrexate-damaged tumour cells. This would account for the clinical phenomenon of apparent tumour specificity.

The number of patients reported in this study is too small to permit dogmatic conclusions. It is possible that the cellular stromal infiltrate is coincidental to the susceptibility of the tumour to methotrexate.

In a series of 161 patients with a solid tumour treated by methotrexate, Sullivan *et al.*[3] reported that 52 (32.2%) had an objective response. It has been shown that 33 of 110 patients had a moderate to marked mononuclear cell response in the tumour stroma (30%).[5] Thus it is possible that in Sullivan's series those patients who responded would have such a round-cell stromal infiltrate. In two series of patients with epidermoid carcinoma of the head and neck the total number of cases treated was 50;[8, 17] of these, 29 (58%) had tumour regression. Table IV presents an analysis of 82 cases of epidermoid carcinoma of the head and neck from the Canadian Tumour Reference Centre. Fifty-one of these (62%) had a moderate to marked round-cell infiltrate of the tumour stroma. Obviously an analysis of the cellular infiltrate of the tumour stroma in a large number of patients treated with methotrexate would be needed to confirm the correlation between response and infiltrate, but these data indicate that the overall percentage of cases with a stromal infiltrate in an unselected group corresponds with the percentage of those who had a response to methotrexate. While this evidence is circumstantial, it at least allows the suggestion that a correlation may exist.

TABLE IV.—FREQUENCY OF STROMAL INFILTRATE BY LYMPHOCYTES AND PLASMA CELLS IN 82 CASES OF EPIDERMOID CARCINOMA OF HEAD AND NECK—THE CANADIAN TUMOUR REFERENCE CENTRE

	Absent or minimal	*Moderate to marked*
Gingival mucosa	2	
Skin of scalp	1	2
Skin of ear	3	7
Lip	5	18
Buccal mucosa	2	4
Tongue	7	1
Pharynx and larynx	3	4
Skin of face	5	13
Vocal cord	3	
Skin of neck		2
	31	51
%	38	62

May the reduction in tumour size be due to the depletion of the stroma of mononuclear cells rather than to a direct reduction in the number of tumour cells? Some reduction in size must be due to this, but it is difficult to decide how much this would contribute to shrinkage of the tumour. If it were significant, one would anticipate that other immunosuppressive drugs would bring about a similar reduction in tumour size. High doses of steroids or purine antagonists are expected to reduce the stromal mononuclear infiltrate, yet these drugs have not effected regression in solid tumours. Although the question remains open, our impression is that this depletion is not a major factor in the reduction of tumour size.

It is probable that the intra-arterial infusion of high doses of methotrexate in regional chemotherapy is directly cytotoxic to the tumour cells and that in these circumstances the relationship described above might not be seen. An important point is that there should not be too long an interval between the biopsy of the tumour and treatment with methotrexate if a correlation between the stromal infiltrate and the tumour response is to be attempted. Eight months' lapse between biopsy and treatment may allow depletion of the stromal infiltrate, as in the patient R.B., and the correlation would be false. Much is known of the mechanisms whereby leukemic cells become resistant to the action of methotrexate.[1] If the lymphocyte is proved to be an important effector cell in determining methotrexate specificity, as is suggested by the present study, then it is possible that development of resistance could be prevented, theoretically, if it were possible to cause a repopulation of the depleted tumour stroma in the recovery period following a course of methotrexate.

Summary A patient with widely metastasizing epidermoid carcinoma of the lung had a dramatic but transient response to treatment with oral methotrexate. He had shown a delayed hypersensitivity reaction to acellular extracts of the tumour. The pre-treatment biopsy revealed a dense stromal infiltrate with lymphocytes and plasma cells and there was an 80% regression of his tumour. A second biopsy showed a moderate stromal infiltrate with mononuclear cells and there was a 50% regression of the tumour following administration of methotrexate. When there was a further recurrence of the tumour, a third biopsy showed absent lymphocytes and plasma cells in the stroma and on this occasion there was no objective regression produced by methotrexate. A review of the pathologic material of 10 patients with tumours treated by methotrexate, without knowledge of the clinical course, allowed a correct prediction of response in seven, based on the presence or absence of a mononuclear cell stromal infiltrate. Six other patients have been studied and the overall score in correct prediction of response to methotrexate is 75%. The implications of these findings are discussed, with reference to possible immunochemotherapy.

Résumé Un traitement *per os* au méthotrexate a donné des résultats spectaculaires, quoique temporaires, chez un malade souffrant de multiple métastases d'un cancer épidermoïde du poumon. Ce malade avait présenté une réaction tardive d'hypersensibilité aux extraits non cellulaires de la tumeur. La biopsie préthérapeutique avait révélé un infiltrat dense du stroma, avec lymphocytes et cellules de plasma, et la tumeur avait régressé de 80%. Une deuxième biopsie avait montré un infiltrat modéré du stroma avec cellules mononucléaires. Le traitement au méthotrexate avait fait régresser la tumeur de 50%. Une troisième biopsie, pratiquée à l'occasion d'une nouvelle récidive de la tumeur, montra que le stroma ne contenait ni lymphocytes ni cellules plasmatiques et, cette fois, le méthotrexate n'entraîna pas de régression objective de la tumeur. L'étude des spécimens pathologiques obtenus chez 10 patients porteurs de tumeurs et traités au méthotrexate, en l'absence de toute donnée concernant l'évolution clinique, a montré qu'on avait pu prévoir correctement l'issue du traitement chez sept malades, selon qu'il y avait présence ou absence d'infiltrat de cellules mononucléaires dans le stroma. Nous avons observé six autres malades: la cote globale de prévision correcte de la réaction au méthotrexate était de 75%. Les répercussions de ces constatations sont examinées, notamment concernant la possibilité de l'immunochimiothérapie.

Thanks are expressed for the technical assistance of Miss T. Satin in the preparation of the material for skin testing and to Dr. R. Heringer for performing the biopsies on R.B. We are indebted to Dr. D. Magner and Dr. M. O. Klotz for their generosity in making readily available all pathological specimens and reports. We thank the Photographic Department of the University of Ottawa for the photographs.

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